MAIL-STOP AMENDMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of MICHAEL ECONS, *et al.*	: Group Art Unit: 1647
Appln. No.:	09/901,938	: Examiner: C. Saoud
Filed:	July 10, 2001	: Attorney Docket No. 053884-5001
For:	NOVEL FIBROBLAST GROWTH FACTOR (FGF23) AND METHODS OF USE	:

DECLARATION UNDER 37 C.F.R. § 1.131

We, Michael Econs, Kenneth White, Tim Matthias Strom and Thomas Meitinger, declare as follows:

1. We are the named co-inventors of claims 3, 4, 12 and 33 in the above-referenced U.S. Patent Application (09/901,938).

2. According to the Examiner, the reference of Itoh et al. (U.S. Patent Application Publication No. US 2002/0082205 A1; "Itoh") discloses an amino acid sequence, "SEQ ID NO:4," that is 100% identical to SEQ ID NO:2 of the above-referenced patent application, and further, claims a nucleic acid encoding an amino acid sequence that is 100% identical to SEQ ID NO:2 of the above-referenced patent application.

3. According to the Examiner, the reference of Milne-Edwards et al. (U.S. Patent Application Publication No. US 2002/0102604 A1; "Milne-Edwards") discloses an amino acid sequence, "SEQ ID NO:298," that is 100% identical to SEQ ID NO:2 of the above-referenced patent application, and further, claims a nucleic acid encoding an amino acid sequence that is 100% identical to SEQ ID NO:2 of the above-referenced patent application.

4. The Office Action states that Itoh and Milne-Edwards therefore anticipate claims 3, 4, 12 and 33 of the above-referenced patent application, under 35 U.S.C. § 102(e).

5. The attached documents in Exhibit A disclose isolation of a nucleic acid (SEQ ID NO:1) encoding an FGF23 polypeptide, the amino acid sequence of which is set forth in SEQ ID NO:2 of the above-referenced patent application.

6. Isolation of the FGF23 nucleic acid (SEQ ID NO:1) encoding the amino acid sequence set forth in SEQ ID NO:2 of the above-referenced patent application, as illustrated in Exhibit A, was conducted in the United States on a date before December 8, 1999. Accordingly, the subject matter of pending claims 3, 4, 12 and 33 was invented prior to the earliest priority dates of December 8, 1999, and March 8, 2000, of the cited references of Milne-Edwards et al. (U.S. Patent Application Publication No. US 2002/0102604 A1) and Itoh et al. (U.S. Patent Application Publication No. US 2002/0082205 A1), respectively.

7. We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Respectfully submitted,

Date: 13 Dec 2004 By: _____
Michael Econs

Date: 13 Dec 2004 By: _____
Kenneth White

Date: 15/Dez/2004 By: Tim Strom
Tim Matthias Strom

Date: 15/Dez/2004 By: T. Meitinger
Thomas Meitinger

Exhibit A

Invention Disclosure as
Evidence of conception of present invention
prior to December 8, 1999

Submitted in conjunction with
the Amendment filed on JANUARY 14, 2005,
which responds to the Office Action dated July 23, 2004 (Paper No. 072104), mailed in connection with U.S. Patent Application No. 09/901,938

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CERTIFICATION PAGE

Title of Invention:

Identification and cloning of the gene responsible for Autosomal Dominant Hypophosphatemic Rickets (ADHR).

Inventor(s):

Michael Econs MD

Signature | Date

Michael J. Econs, M.D., Associate Professor of Medicine and Medical | United States and Molecular Genetics

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Kenneth E. White, Ph.D.

Signature | Date

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Witness(es):

I certify that the invention has been explained to and is understood by me.

Steven Waguespack, M.D.

Printed Name in Full | Date | Signature

Home Address | Home Phone | (317)278-0668 Business Phone

C. Conrad Johnston, Jr., M.D.

Printed Name in Full | Date | Signature

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Invention Disclosure Form

Identification of the gene responsible for Autosomal Dominant Hypophosphatemic Rickets (ADHR).

Inventors: Michael Econs, MD; Ken E. White, PhD; Tim M. Strom, MD (Munich); Thomas Meitenger MD (Munich); Jeffrey O'Riordan, MBBS of University College London provided a family and Harald Juppner, MD of Mass General Hospital provided a library.

ADHR Invention description:

1. Prior to our discovery, the gene that is responsible for the disorder autosomal dominant hypophosphatemic rickets was unknown.

2. There have not been other attempts to find this gene. However, numerous investigators have tried, unsuccessfully, to identify the phosphaturic factor that we have called "phosphatonin" (1).

3. Description of the invention:

Linkage:

We mapped the ADHR gene locus to an 18 cM region of chromosome 12p13 through a genome-wide linkage screen performed with microsatellite repeat markers on a large ADHR kindred, family 1406 (2). To refine the ADHR interval, we performed linkage analysis with an additional 12-15 markers in an effort to saturate the ADHR region. In family 1406, haplotype analysis detected a recombination event between the ADHR gene and the marker D12S1685, which is now the closest telomeric marker (manuscript in preparation). In agreement with this finding, another ADHR kindred, family 1478, showed a recombination event at D12S1050, slightly telomeric to D12S1685. On the centromeric side of the ADHR gene, D12S397 continued to be the closest flanking marker in family 1406, whereas family 1478 recombines at D12S1594, thus defining the closest proximal marker. Based upon these studies, the ADHR candidate interval was between D12S1685 and D12S1594, a region of approximately 1.5 Mb. We used the publicly available Einstein College of Medicine 12p13 physical map as well as the Marshfield chromosome 12 map to establish a new marker order of: tel-D12S100-D12S1050-D12S1685-D12S1725-D12S1594-D12S314-CD4-D12S397-cent.

Identification of exons:

After localizing the ADHR gene locus to 1.5Mb on chromosome 12p13, we sought to identify genes that are located between the flanking markers. We obtained bacterial artificial chromosome (BAC) sequence from the region (BAC 388F6, GenBank accession no. AC008012) from the Baylor College of Medicine

sequencing center (http://www.hgsc.bcm.tmc.edu/) as part of the Human Genome Project. To determine if exons with appropriate splice acceptor and donor sites were present in 388F6, the exon prediction program GenScan (http://CCR-081.mit.edu/GENSCAN.html) was used after the raw sequence was masked for repeat sequences using Repeatmasker (http://repeatmasker.genome. washington.edu/cgi-bin/RepeatMasker). GenScan predicted 3 novel, contiguous exons encoding a 753 bp ORF (followed by an in-frame stop codon) that possessed approximately 30-40% homology to the fibroblast growth factor (FGF) family. The gene spans at least 10 kb of genomic sequence. No sequences encoding genes or expressed sequence tags (ESTs) identical to the predicted exons were found after BLAST searches (http://www.ncbi. nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform=0) of GenBank and the dbEST. We designated this novel peptide "FGFL" or "fibroblast growth factor-like protein".

To test if the predicted exons indeed formed an expressed FGFL mRNA, we performed RT-PCR with human heart RNA using primer sets targeting predicted exons 1 and 3. This approach produced an expected cDNA of 610 bp, with exact sequence identity to the predicted FGFL sequence within this region, thus verifying that FGFL was an expressed gene. We then performed the same procedure on an arrayed tumor induced osteomalacia (TIO) cDNA library to determine if the transcript was present within the library. Out of 88 pools with 1000 clones per pool, 10 positive pools were identified, indicating that FGFL mRNA was expressed by the tumor. To identify the 5' UTR, we performed PCR on a positive TIO pool with an FGFL-specific primer targeting exon 1 and a vector-specific SP6 primer. After sequence analysis of the resulting cDNA products, 146 bp of 5' UTR was identified in the clone. To amplify the 3' UTR of the cDNA, an FGFL-specific primer located within exon 3 and the vector-specific T7 primer were used on the same TIO pool. DNA sequencing of the PCR product revealed that the clone contained 338 bp of predicted 3' UTR, followed by a poly $(A)^+$ tail. The cDNA sequence from the library was aligned with the genomic DNA sequence and the intron-exon boundaries were determined. Exon 1 is comprised of at least 146 bp of 5' UTR and 211 bp of coding sequence, exon 2 is 104 bp, and the third exon is 441 bp of coding sequence (including stop codon) and 331 bp of 3'UTR. The first nucleotide of the start codon, analyzed with the ATGpr program (http://www.hri.co.jp/atgpr/), was predicted to be at nucleotide 147 by analysis of potential Kozak consensus sequences (consistent with the prior GenScan prediction). The sequence surrounding the start codon was ACGATGT, in high agreement with the consensus sequence of A/GXXATGG. The program SignalP V2.0 (http://www.cbs.dtu.dk/services/SignalP/) was then used to determine if FGFL contained a potential signal peptide sequence. The analysis under the two models used by SignalP indicated that a signal peptide existed with a probability of 0.994 and 0.990, with cleavage of the peptide most likely between the alanine residue at position 24 and the tyrosine at position 25. In summary, FGFL is

expressed as a mature mRNA in both a normal tissue as well as a tumor that leads to phosphate wasting, and is comprised of a 146 bp of 5' UTR, a 756 bp ORF (251 amino acids), followed by 338 bp of 3'UTR and a poly(A)$^+$ tail. FGFL contains a high probability start codon and a predicted cleavable signal peptide.

While we were performing the above analysis, our collaborator, Dr. Strom was performing 5' RACE and 3'RACE and obtained similar results.

Mutation detection:

Mutation analysis of the three FGFL exons comprising the coding region was directly performed by sequence analysis of 40 patients with various hypophosphatemic disorders. Briefly, genomic DNA was amplified by PCR using primers that spanned each FGFL exon, taking caution to include analysis of the splice donor and acceptor sites, the cDNAs were purified by spin chromatography, and sequenced using either the forward or reverse PCR primer. Several common polymorphisms were detected, however two heterozygous missense mutations were not detected in an initial screen of 30 control individuals. These changes were nucleotide G547A, which leads to residue change R176Q, and C555T, which results in the missense R179W. The R176Q mutation occurs in the two hypophosphatemic families, 1406 and 1478, that were used to localize and to narrow the ADHR region on chromosome 12p13 (see above). The G547A mutation destroys an AciI site in the cDNA, therefore PCR primers were designed to flank the region surrounding the mutation. RFLP analysis of families 1406 and 1478 by AciI digestion showed that the mutation segregated exactly with the disease haplotype in both families. The control PCR product was digested into fragments of 112 bp, 49 bp, and 33 bp, whereas the mutant alleles only produced 112 and 82 bp fragments, when analyzed by agarose gel electrophoresis. Over 400 control individuals were tested for the mutation, with none of the control samples containing the aberrant RFLP pattern.

The upstream missense, R179W, occurs in a hypophosphatemic family, family 2318, previously excluded from chromosome Xp22 (the X-linked hypophosphatemic rickets interval) by linkage analysis (3). The C555T change creates a BpmI restriction site. The same primer pair used above for RFLP analysis was used to amplify the region surrounding the C555T change. BpmI digests demonstrated that the R179W missense segregated with the disorder in family 2318. The PCR product from the normal allele was not digested, leaving the original product of 194 bp, whereas the mutant alleles were cut a single time and resulted in 118 and 87 bp fragments, when analyzed by agarose gel electrophoresis. An additional 367 controls were analyzed and no individuals were identified with the aberrant restriction pattern, thus confirming the change as a mutation.

4. We are the first group of investigators to find the gene responsible for ADHR. Homology searches indicate that it is a member of the fibroblast growth factor family. The gene has a signal peptide sequence, which indicates that it is secreted. It is likely that the protein alters phosphate uptake in the renal proximal tubule and plays a role in normal phosphate homeostasis.

5. We have identified the gene and have identified key regions, which when mutated result in renal phosphate wasting.

6. Based on the fact that mutations of this gene result in phosphate wasting, this gene probably plays an important role in normal renal phosphate homeostasis. The deduced protein structure indicates that it has a signal peptide and, therefore, is secreted. Since all of the mutations that we have found to date are missense mutations, these mutations could be either activating mutations in a gene that codes for a phosphate wasting hormone or inactivating mutations in a gene that codes for a protein that functions as a phosphate conserving hormone.

If the protein codes for a phosphate wasting hormone, or "phosphatonin", the product could be used to treat the hyperphosphatemia that accompanies renal insufficiency. If the protein codes for a phosphate conserving substance the protein would be useful to treat disorders of renal phosphate wasting, such as autosomal dominant hypophosphatemic rickets, X-linked hypophosphatemic rickets, tumor induced osteomalacia and hereditary hypophosphatemic rickets with hypercalciuria.

Since the protein may be important in paracrine actions on bone, either the protein, an analogue to the protein, or a pharmaceutical agent that alters expression of the protein, may have effects on bone density and structure and could be used as a therapy for osteoporosis.

Additionally, the sequence can now be used to identify mutations in patients with phosphate wasting as a diagnostic test. The protein can also be used to identify and clone its receptor. Antibodies to this protein would be useful for a serum assay to detect serum levels of this protein in various bone diseases. The gene can be used to identify the mouse homologue and create knockout and "knockin" mice.

Supporting Information:
1. See #6, description, above.

2. There are no known competitive products. However, in 1995 we identified a gene, PEX (now referred to as "PHEX") that is responsible for the disease X-linked hypophosphatemic rickets. We did not patent that gene. One year after publication of our findings Karapalis et al submitted a patent application (included) on the PHEX protein. PHEX codes for an enzyme.

3. Many biotech firms. Amgen may have a specific interest since they are developing bone active agents. Diagnostic companies, such as Quest

Diagnostics and Boehringer (now Roche) may be interested in developing serum assays.

4. We have not published or otherwise publicly disclosed these findings.

5. We have numerous plans for further research. We plan to express the protein and see its effect on phosphate transport. We may make a knockout mouse. We will make antibodies to this protein to further study expression and we would like to develop a serum assay for this protein.

6. We have an email identifying base pair changes in two of our patients. We have multiple other emails between members of the collaboration. We have laboratory records for mutation detection, identification of the 5' and 3' UTRs.

7. There is very little recent literature about this disease. The disease was first described in the 1970s. We further characterized the disease and mapped the gene's location to chromosome 12p13 (2). We have further refined the location of the disease gene (4). The gene has not been identified by other investigators. We have not searched the patent database for sequences that may be patented by companies that are sequencing and patenting ESTs.

8. These investigations have been funded by NIH grants R01 AR42228 and K24 AR 02095 to Dr. Econs and F32 AR08550 to Dr. White. This work was not industry sponsored. Dr. Econs has a VA appointment, but receives no money from the VA (WOC) and this work was not VA sponsored. Drs. Meitenger and Strom, presumably, have funding from the German government.

References:

1. Econs, M. J. and Drezner, M. K. (1994) Tumor-induced osteomalacia-unveiling a new hormone. *New Engl J Med* 330, 1679-1681.

2. Econs, M. J., McEnery, P. T., Lennon, F., and Speer, M. C. (1997) Autosomal dominant hypophosphatemic rickets is linked to chromosome 12p13. *J Clin Invest* 100, 2653-2657.

3. Rowe, P. S. N. (1994) Molecular biology of hypophosphataemic rickets and oncogenic osteomalacia. *Hum Genet* 94, 457-467.

4. White, K. E., Speer, M. C., Biber, J., Murer, H., and Econs, M. J. (1998) Refining the autosomal dominant hypophosphatemic rickets (ADHR) interval on chromosome 12p13 and localization of two candidate ADHR genes. *Bone* 23 (5), S379.(Abstract)

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CERTIFICATION

I, Michael Econs, M.D., hereby certify that the invention entitled Idendification and cloning of the gene responsible for Autosomal Dominant Hypophosphatemic Rickets (ADHR). patent application no. Pending, was made by me on _____, ___, while I was ~~employed~~ without compensation at the VA Medical hospital priviledges Center, _____, while employed as (Title of Position):_____. The other inventor(s) were: Kenneth White, Tim Strom, Thomas Meitinger.

The invention was made:

1. During official working hours: Yes ____ No X

2. With a contribution by the VA of:
   - (a) Facilities — Yes ____ No X
   - (b) Equipment — Yes ____ No X
   - (c) Materials — Yes ____ No X
   - (d) Funds — Yes ____ No X
   - (e) Information — Yes ____ No X
   - (f) My time or services while on official duty — Yes ____ No X
   - (g) Time or services of other VA employees on office duty — Yes ____ No X

3. The invention:
   - (a) Bears a direct relation to my official duties — Yes ____ No X
   - (b) Was made in consequence of my official duties — Yes ____ No X

4. I am attaching remarks relating to the above — Yes ____ No X

Signatures: _____
(Immediate supervisor)

Signature: _____
(Inventor)

Present Title: Associate Professor of Medicine and Medical and

Home Address: _____

ENTERED

Addendum

Please note that I have had a "without compensation" appointment at the Roudebush VA Medical Center since coming to Indiana University School of Medicine
I do not have official VA working hours nor an official VA tour of duty. I do, however, have hospital privileges at the VA Medical Center. This work was done totally independent of any VA resources, personnel, or facilities.

Michael J. Econs, M.D., F.A.C.P., F.A.C.E.
Associate Professor of Medicine and
Medical and Molecular Genetics.